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Arsenic Distribution and Speciation near Rice Roots Influenced by Iron Plaques and Redox Conditions of the Soil Matrix

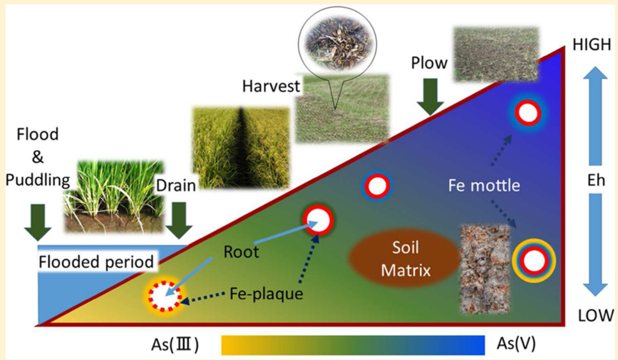
Noriko Yamaguchi,[†,*] Toshiaki Ohkura,[†] Yoshio Takahashi,[‡] Yuji Maejima,[†] and Tomohito Arao[†]

[†]National Institute for Agro-environmental Sciences, 3-1-3, Kan-nondai, Tsukuba, 305-8604, Japan

[‡]Department of Earth and Planetary Systems Science, Hiroshima University, Kagamiyama, Higashi-Hiroshima, Hiroshima 739-8526, Japan

S *Supporting Information*

ABSTRACT: Elevated arsenic (As) concentrations in rice and the soil solution result from changes in soil redox conditions, influenced by the water management practices during rice cultivation. Microscale changes in redox conditions from rhizosphere to soil matrix affect the As speciation and Fe plaque deposition. In order to focus on the rhizosphere environment, we observed microscale distribution and speciation of As around the rhizosphere of paddy rice with X-ray fluorescence mapping and X-ray absorption spectroscopy. When the soil matrix was anaerobic during rice growth, Fe-plaque did not cover the entire root, and As(III) was the dominant arsenic species in the soil matrix and rhizosphere. Draining before harvest led the conditions to shift to aerobic. Oxidation of As(III) to As(V) occurred faster in the Fe-plaque than the soil matrix. Arsenic was scavenged by iron mottles originating from Fe-plaque around the roots. The ratio of As(V) to As(III) decreased toward the outer-rim of the subsurface Fe mottles where the soil matrix was not completely aerated. These results provide direct evidence that speciation of As near rice roots depends on spatial and temporal redox variations in the soil matrix.



■ INTRODUCTION

Chronic arsenic (As) exposure from food and water is carcinogenic to humans.[1] Paddy rice contains greater As concentrations because it is mainly grown under flooded conditions.[2] When a soil is aerobic, As is less mobile because arsenate (As(V)) is strongly sorbed to mineral soil components, such as Fe and Al (hydr)oxides.[3] When anaerobic conditions develop in flooded soils, As(V) is reduced to arsenite (As(III)) in the soil solid phase.[4−6] Then, As(III) is desorbed from soil minerals due to a lower sorption ability compared to As(V).[3] In addition, Fe (hydr)oxides, which are ubiquitous in paddy soils, are subjected to reductive dissolution due to Eh drop in combination with the activity of Fe-reducing bacteria.[7,8] Consequently, the arsenic associated with Fe (hydr)oxides is released into the solution as the adsorption phase is lost.[9] Larger amounts of As are dissolved in soil solution under anaerobic conditions, which leads to greater As concentrations in rice grain.[10,11]

Even in the predominantly anaerobic soil matrix, the rhizosphere of rice becomes aerobic due to radial oxygen loss (ROL) through the root aerenchyma to the root surface.[12−14] The rate of ROL is different between genotypes[15−18] and is generally greater for rice cultivars with greater root porosity.[18] The ROL followed by rhizosphere oxygenation as well as the reductive dissolution of Fe-bearing minerals in the soil causes the formation of Fe-plaque around the roots. Fe-plaque is composed of ferrihydrite, goethite, and lepidocrocite.[19−22] These Fe minerals strongly adsorb As[3,23−25] and function as an As barrier for the root.[26−28] The amounts of Fe-plaque and sequestrated As increase on the root surface with increasing rates of ROL.[15] Significant correlations between the spatial distributions of As and Fe near wetland plant roots indicate that As sequestration in Fe-plaque occurs.[29,30] It was reported that sequestrated As was mainly composed of As(V) with only a small presence of As(III),[30,31] despite the fact that As in the soil solution was composed mainly of As(III). The oxidation of As(III) to As(V) may be facilitated in the rhizosphere by the greater O_2 concentration and the presence of Fe^{2+} via the Fenton reaction, which catalyzes the oxidation of As(III) in the aqueous phase and the As(III) sorbed on Fe minerals.[32−34] Since Fe-plaque on rice roots has a greater affinity for As(V) than As(III),[35] oxidation of As(III) in the rhizosphere promotes the sequestration of As in Fe-plaque.

When rice was grown under anaerobic conditions, Fe-plaque coatings were not observed on young rice roots or on the younger portions of the matured roots, which are the most active areas of solute uptake, whereas distinct pigmentation by

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Fe-plaque was found on mature roots.[36] The authors reported that Fe-plaque does not directly restrict As absorption by roots but predominantly immobilizes As near the root base. Conversely, Liu et al.[31] identified greater Fe-plaque formation and As accumulation on the root tips than on the root base, which potentially resulted from increased ROL toward the root tip.[37] Although Seyfferth et al.[36] stated that this contrasting tendency for Fe-plaque deposition potentially resulted from genotype differences, different redox conditions during rice cultivation may also cause such differences. For example, Seyfferth et al.[36] cultivated rice directly in flooded soil whereas Liu et al.[31] cultivated rice in glass beads that were packed in nylon mesh and surrounded by soil.

The formation of Fe-plaque is influenced not only by the morphological and physiological properties of the plant, but also by various soil-derived factors such as Fe and Mn concentrations.[20,38] For example, the amount of Fe-plaque per root weight is greater in flooded soil than in aerobic soil because greater amounts of Fe(II) are produced by the reductive dissolution of Fe minerals in the soil matrix and supplied to the rhizosphere.[39] The redox conditions of paddy soils are spatially and temporarily dynamic due to their heterogeneous soil oxygen concentrations.[13,40] This heterogeneous oxygen distribution results from microbial activity, root distribution, and soil morphology. In flooded soil profiles, the redox potential decreases with depth.[40,41] Thus, Fe-plaque was found mostly on roots near the surface of the flooded soil.[36,40] Conversely, Fe-plaque was less prevalent in the subsurface anaerobic zone, especially after prolonged flooding.[40] In addition, the formation of Fe-plaque is associated with rice growth stage. Fe-plaque formation decreases during the late stage of rice growth,[13,21,40] which is associated with increasing As concentrations in rice stems.[42] In contrast, Mei et al.[15] suggested that As sequestration in Fe-plaque is greater in the late stage of rice growth.

Draining flooded water allows diffusion of O_2 into the soil thereby allowing a fast soil matrix shift to aerobic conditions near the surface, while it is slower in the deeper soil layer.[41] In the soil matrix, the development of aerobic condition is much faster than that of strongly reducing condition.[41] Even after shrinkage and decomposition of roots, Fe mottles originating from Fe-plaque around roots remain along with channel walls. In addition to the root-derived Fe mottles, those originating from deposition of Fe oxide around air-filled channels and entrapped air in voids were present in the soil after drainage.[43] The mineral composition of Fe mottles varies depending on the forming condition. Root-derived Fe mottles include well-crystallized lepidocrocite, whereas Fe mottles on soil pedon surfaces are dominated by poorly crystallized Fe-oxides.[44] The inhibition of crystalline Fe mineral formation, which occurs in the Fe mottles on soil pedon, resulted from the adsorption or incorporation of Si and P.[44,45] The Fe mottles that remain after root decomposition can potentially scavenge As between rice cultivation flooding periods because the Fe-plaque formed during rice cultivation scavenges As;[16,17,22,26,29−31,35,36,38] however, direct evidence of As sequestration in Fe-mottle has not been reported. If As is sequestrated, then the crystal growth of Fe-hydroxide to form Fe-mottle would be inhibited.[46,47] The Fe-mottle can also scavenge As from the pore water.

Variable water management associated with the growth stages of rice is important for improving rice grain yield and quality. In a typical cultivation cycle of paddy rice in Japan, the soil is flooded for 5 months, but fields are drained twice: once for 2 weeks 40 days post-transplant, and once 10 days prior to harvest. Consequently, paddy soils undergo repetitive wetting and drying cycles that result in spatially and temporally variable redox conditions. It is well-documented that the water management practices influence As concentration in soil solution and rice grains.[10,11] Nonetheless, the relationships between water management, which controls redox condition of the soil matrix (i.e., bulk soil, not including the rhizosphere), and the distribution and speciation of As in the rhizosphere are poorly understood. A detailed investigation of the rhizosphere soil requires an analytical technique that allows for micrometer spatial resolution. Therefore, we utilized synchrotron microbeam X-ray fluorescence mapping (μXRF) combined with μ-X-ray absorption near edge structure (μXANES). This study aimed to clarify the effects of the soil redox conditions of the soil matrix on the distribution and speciation of As and Fe in the rhizosphere of rice by considering the water management of paddy rice cultivation. We investigated the relationships between the distribution and speciation of As and Fe-plaque when the soil matrix was (1) anaerobic, (2) in transition from anaerobic to aerobic, and (3) under aerobic conditions. The role of residual Fe-mottles, which remained after the rice cultivation period, on As sequestration was also investigated.

■ MATERIALS AND METHODS

Soil Samples. Soil samples were obtained from experimental paddy fields in Japan. The total As concentrations in the plowed soil layer (0−15 cm) of paddy fields A and B were 0.93 and 0.49 mmol kg^{-1}, respectively. The total As concentration in soil A was higher than the background As concentration in Japanese soil,[48] 0.016 − 0.51 mmol kg^{-1} due to the past inflow of contaminated irrigation water from a mining area. The distance between fields A and B is 20 km. Both soils were classified as *Aeric Epiaquepts* according to US taxonomy.[49] All relevant soil properties are presented in Supporting Information (SI) Table S1.

Preparation of Soil Thin Sections. Soil thin sections with rice roots or Fe mottles were prepared with reference to the various soil redox conditions associated with the water management of the paddy fields. Three different management periods were considered, including periods of rice plant growth with soil flooding (anaerobic conditions), 1 month after water drainage and rice harvest (transitional period from anaerobic to aerobic conditions), and 6 months after harvest (aerobic conditions in plow layer).

Thin sections of the soils under anaerobic condition with rice roots were obtained from pot experiments. *Oryza sativa*, cv. Koshihikari were grown in pots with 2.5 kg of soil from paddy fields A or B. Soil in the pots was mixed with water to mimic the puddling process of paddy field before transplanting the rice seedlings. Next, the soil surface was flooded continuously for 35 days, then drained and not flooded for 3 weeks and flooded again. One week after panicle exertion (10 days after the second flooding), the soil redox potential at a depth of 10 cm from the soil surface was measured by a Eh meter (PCM 90, TOKO, Tokyo, Japan). Subsequently, wet soil blocks (10 × 10, 20 cm in depth) that included the shoot base and roots were cut out with a knife. The soil was not drained during this process to maintain anaerobic soil conditions. Next, each soil block was cut into 3 cm thick blocks and immediately frozen in liquid nitrogen. A portion of the soil block that contained roots was freeze-dried to determine the As and Fe concentrations, as described below.

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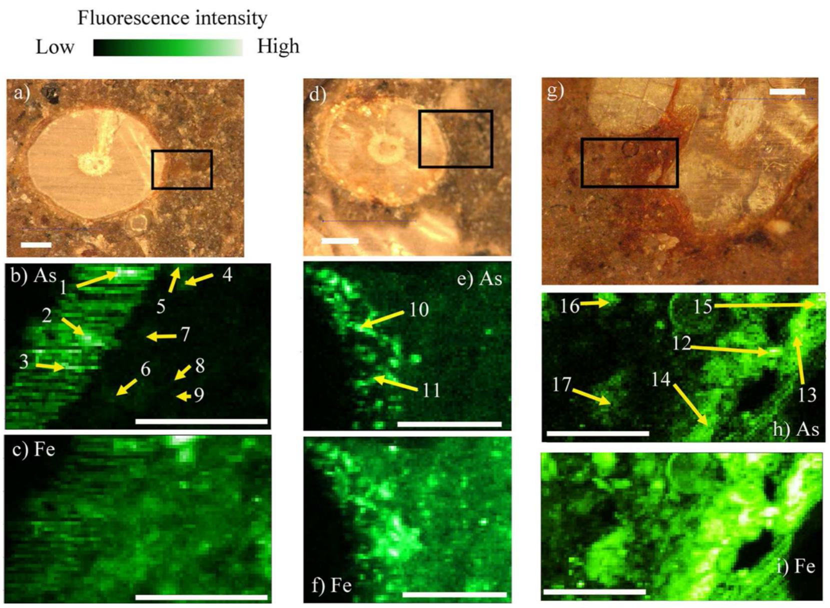

Figure 1. Light microscope and micro X-ray fluorescence (μXRF) images around the rice roots when the soil was flooded. Root with a visible Fe-plaque coating from soil A (a−c), root without a visible Fe-plaque coating from soil A (d−f), and root with visible Fe-plaque coating from soil B (g−i). Light microscope images (a, d, g), total As (b, e, h), and total Fe (c, f, i) images. Numbers indicate points where the As K-edge μXANES in SI Table S3 and Figure S5 were obtained. Black boxes in (a, d, g) indicate where the μXRF maps were obtained and white bars indicate 200 μm.

Thin sections of the soil after harvest were prepared from a soil cylinder (80 mm in diameter × 30 cm in depth) collected from paddy field A in October, 1 month after harvest. The shoot base was withered, but the roots were alive. Soil cylinders were taken to include the shoot base and roots. Flooded water was drained before harvest, and the paddy soil had not been flooded for approximately 1 month. Soil cylinders were cut into sections 3 cm in height and frozen with liquid nitrogen.

In April, 6 months after harvesting, soil cylinders (50 mm in diameter × 30 cm in depth) were taken from paddy fields A and B. The soil had not been flooded for approximately 7 months and the soil surface was plowed to incorporate the shoot base and roots. Soil cylinders were sliced into blocks 3 cm in thickness and frozen with liquid nitrogen.

The above-mentioned frozen soil blocks were freeze-dried, embedded in epoxy resin, and cut into thin sections with a thickness of approximately 80 μm, as described in the SI. Freeze-drying and embedding in epoxy resin caused negligible changes in As speciation (SI Figure S1).

Microscale Distribution and As and Fe Speciation. Microscale As and Fe distributions in soil thin sections were determined by μXRF at beamline 4A of the Photon Factory in KEK and at BL10.3.2 at the Advanced Light Source (ALS). Chemical speciation maps for As(III) and As(V) were obtained at BL10.3.2 at ALS. Arsenic K-edge μ-X-ray absorption near edge structure (μXANES) spectra were collected from the spots of interest in the thin section, based on the μXRF map near the soil-root interfaces and on the soil particles. The Fe mineral phases in the selected points on Fe mottles were identified from micro-X-ray diffraction (μXRD) patterns at an incident energy of 17 keV (= 0.07293 nm) with a CCD camera at ALS BL10.3.2 and Fe K-edge μXANES. A description of the experimental setup and data analyses is included in the SI.

As and Fe Extraction from the Soils and Roots. Freeze-dried soils and roots, obtained from the pots after collecting soil block samples for thin sections, were further analyzed to determine concentrations of As and Fe associated with poorly crystallized Fe hydroxides. Roots with and without Fe-plaque stains were removed from the soil separately with tweezers following visual inspection. In addition, visible soil particles attached to the roots were removed with tweezers. To determine the concentrations of As and Fe associated with poorly crystallized Fe-oxides, the roots were extracted with 0.2 M $(NH_4)_2C_2O_4/H_2C_2O_4$ at pH 3 by shaking for 4 h in the dark and then filtered by a 0.20 μm membrane filter.[50] The As and Fe concentrations in the filtrate were determined with an inductively coupled plasma optical emission spectrometer (ICP-OES, VistaPro, Varian, Palo Alto, U.S.). The roots with and without Fe-plaque staining were analyzed separately.

RESULTS

Distribution and Speciation of As around Roots during the Growth of Rice Plants in Flooded Soils. When the soil blocks with rice roots were collected, the soil redox potential (Eh) and pH at a depth of 10 cm from the soil surface was −90 mV and pH 7.0 for soil A and +213 mV and pH 6.8 for soil B, respectively. The Eh monitoring data for soil A are shown in SI Figure S2. Although the experimental conditions were the same for both soils, the greater Eh of soil B potentially resulted from its lower organic matter content (SI Table S1). The concentrations of As associated with poorly crystallized Fe hydroxides in the root with the Fe-plaque were greater than As concentrations in roots less stained for both soils (SI Table S2). The ratios of As to Fe were greater in the Fe-plaque than in the poorly crystallized Fe minerals in the soil matrix. This suggests the As was concentrated in Fe hydroxides around the roots (SI Table S2).

Roots with and without Fe-plaque staining were found simultaneously in the soil thin sections (SI Figure S3a). Arsenic was associated with the Fe-plaque on the root surface rather than with the soil matrix (Figure 1b,e,h). The relationships between the fluorescence intensities of As Kα and Fe Kβ (SI Figure S4) also revealed an accumulation of As on the Fe-plaque for the samples presented in Figure 1a,d; however, the

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accumulation was not clearly observed in the section shown in Figure 1g.

The proportion of As(III) to total As on the Fe-plaque attached to the roots and on the Fe minerals in the soil matrix was determined with linear combination fitting (LCF) of μXANES using spectra of reference compounds: sodium arsenite for As(III) and arsenate sorbed on ferrihydrite for As(V). The As K-edge μXANES spectra and fit results are shown in SI Figure S5 and Table S3. When sodium arsenate was used as a reference of As(V), fit results were not different for the data points shown in Figure 1 (flooded period), while the R-factors which indicate the goodness of fit were larger for the data shown in Figure 2 (1 month after harvest) (SI Table S3). The inclusion of organic As species (monomethylarsonous acid and dimethylarsenic acid) as reference compounds for the LCF did not improve the goodness of fit. Thus, based on the LCF fit it was suspected that organic As species only accounted for a trace amount of As around the roots and in the soil matrix. The As(III) content of the Fe-plaque (72 ± 7% for soil A and 46 ± 3% for soil B) and that in the soil matrix (72 ± 9% for soil A, 31 and 30% for soil B) did not differ (SI Table S3). The As(III) proportions on the Fe-plaque around the roots were similar in roots with and without aerenchyma (SI Figure S6). These roots were found at similar depths from the soil surface and the younger portion of root close to root tip did not have aerenchyma.[51]

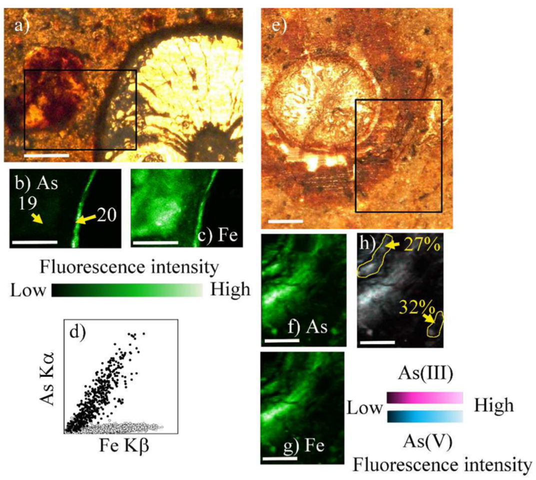

Figure 2. Light microscope and micro-X-ray fluorescence (μXRF) images around rice roots 1 month after harvest in field A. Soil thin sections with roots from well-drained (a–c) and poorly drained locations (e–h). Light microscope (a, e), total As (b, f), total Fe (c, g), As(V), and As(III) chemical speciation (h) images and the relationships between the fluorescence intensities of As Kα (b) and Fe Kβ (c) around roots from the well-drained area (d) are presented. Black and white circles correspond to spots on the Fe-plaque and Fe minerals, respectively (d). Numbers indicate points where the As K-edge μXANES data in SI Table S3 and Figure S5 were obtained. Black boxes in (a) and (e) show where μXRF maps were obtained and white bars indicate 200 μm. Note that fluorescence intensity corresponding to white level of color indicator for bicolor chemical speciation image for As(V) is 3-times higher than that for As(III).

In the roots from rice seedlings grown in pots for 4 weeks under flooded conditions, the Fe-plaque did not coat the root apex. In these samples, As and Fe co-occurred in the root interior. In addition, 80% and 42% of the total As was As(III) in the root interior and on the Fe-plaque, respectively (SI Figure S7).

As Distribution and Speciation around the Roots 1 Month after Harvest. In samples collected directly from the paddy field 1 month after harvest, most of the roots in the soil thin section were stained by Fe-plaque (SI Figure S3b).

In the soil thin section from the well-drained location, roots with aerenchyma had a thin layer of Fe-plaque (Figure 2a). In addition, Fe minerals accumulated at approximately 100 μm from the root (Figure 2a). The fluorescence intensities of the Fe Kβ did not differ between the Fe minerals in the soil matrix and in the Fe-plaque around the roots (Figure 2c). Iron concentrations were proportional to the fluorescence intensities when the sample position and thickness were the same. No peak was observed by μXRD analyses or Fe K-edge μXANES (SI Figure S8) implying that poorly crystallized Fe hydroxide or ferrihydrite dominated both the Fe-plaque and the soil matrix. Similar to the soil thin section prepared from anaerobic soil during rice growth, the fluorescence intensities of the As Kα were greater on the Fe-plaque around the roots than on the Fe aggregates in the soil matrix (Figure 2b). The relationships between the normalized As Kα and Fe Kβ intensities indicated that two clearly different areas occurred regarding the As to Fe ratio (Figure 2d). The areas with greater As to Fe ratios corresponded to areas of Fe-plaque around the roots. The μXANES LCF analysis indicated that 40% of the As associated with Fe minerals 100 μm away from the root surface was in the form of As(III). In contrast, As(V) was the dominant As species on the Fe-plaque attached to the root (Figure 2b, SI Table S3, Figure S5).

In the soil section from the poorly drained location, the root with aerenchyma had Fe-plaque and a concentric Fe accumulation that extended up to 350 μm from the root–soil interface with a diffusive boundary (Figure 2e). The distribution of As followed that of Fe (Figure 2f, g). The distribution patterns of As(V) and As(III) were also similar (Figure 2h). In the area of Fe accumulation within 100 μm from the root surface, the proportion of As(III) was 27 ± 4%, whereas it was 32 ± 5% on the Fe aggregate 300 μm away from the root–soil interface (Figure 2h, area of interest circled by yellow line).

Distribution and Speciation of As and Fe around the Fe Mottles. In samples collected from the paddy field 6 months after harvest, root tissues had decomposed and occasional rusty-colored Fe mottles were observed around pores originating from root channels (SI Figure S3c). Tubular Fe mottles observed at 3–6 and 24–27 cm from the soil surface in soils A and B, respectively, were chosen for analysis since they had different redox conditions based on the field observation of the soil matrix color and on the α,α-dipyridyl test. In the Fe mottles, rusty and orange-colored portions coexisted (Figure 3a,d), and Fe and As co-occurred (Figure 3b,c,e,f), whereas spatial distribution patterns of Fe and As were different.

In the tubular Fe mottles found near the soil surface (Figure 3a–c), the As Kα fluorescence intensity in the outer rim was lower than that of the inner part (Figure 3b). However, the fluorescence intensities of Fe Kβ were similar in both parts (Figure 3c). Thus, the ratio of As to Fe decreased with increasing distance from the root channel.

In the tubular Fe mottles found in subsurface soil (Figure 3d–h), As was localized in the rusty colored portions observed with a light microscope. However, the Fe kβ fluorescence

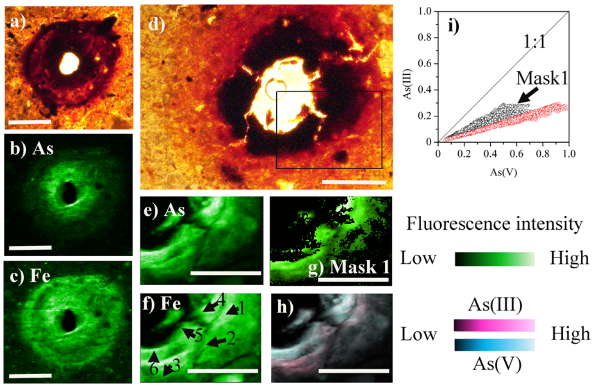

Figure 3. Light microscope and micro X-ray fluorescence images (μXRF) of the Fe mottles at a depth of 3−6 cm from the soil A surface (a−c) and at a depth of 24−27 cm from soil B (d−h). Light microscope (a, d), total As (b, e), total Fe (c, f), and chemical speciation of As(V) and As(III) (h) images are presented. (i) The scatter plots between As(III) and As(V) counts from the chemical speciation map (h) were divided into two regions of different As(III) to As(V) ratios. The data from the higher As(III) region are shown as black open circles. The other data are shown as red open circles. (g) μXRF images for the total As with the masked pixels are shown as black circles in (i). Numbers indicate points where the μXRD data in SI Figure S9 were obtained. The black box in (d) shows where the μXRF maps (e−h) were obtained and white bars indicate 500 μm. Note that fluorescence intensity corresponding to white level of color indicator for bicolor chemical speciation image for As(V) is 3-times higher than that for As(III).

intensity of the rusty and orange-colored areas was similar (Figure 3d,f). The As(III) and As(V) chemical speciation maps indicated that As(III) was generally greater in the outer rim of the tubular mottle than in the inner portion (Figure 3h). The scatter plots of As(V) versus As(III) indicated that the As(III) to As(V) ratio varied. When the pixels with greater As(V) ratios indicated by the red circles in Figure 3i were masked, it was observed that As was located in the outer rim of the Fe mottle (Figure 3g). The data show that As(III) was found on the outer rim associated with lepidocrocite and As(V) in the inner portion associated with goethite and poorly crystallized Fe hydroxide or ferrihydrite (Figure 3h and SI Figure S9).

■ DISCUSSION

When soils are aerobic, As(V) is the dominant species of As. Since As(V) strongly sorbs to Fe minerals,[23] As concentration in soil solution is low.[4,6,10] With the development of anaerobic conditions after flooding, the proportion of As(III) in the soil solid phase is increased followed by the increased concentration of dissolved As(III) and Fe(II).[4,6] Prolonged flooding periods increase the dissolved As(III) concentrations in the soil solution, which results in the increased As concentrations in the rice grains.[10] As(III) in the soil solution is clearly the major source of As in rice grains.

Relationships between As and Fe-Plaque in the Anaerobic Soil Matrix. When rice plants are grown, water and solutes in the rhizosphere move toward the roots, i.e., mobile Fe(II) and As(III) released by reductive dissolution of the host Fe(III) oxyhydroxide minerals are transported toward the root. Under anaerobic soil conditions, As accumulated around the root regardless of the presence of the Fe-plaque observed by light microscope (Figure 1). The contrast of As Kα fluorescence intensity between the root periphery and the soil matrix was greater on roots that had distinct Fe-plaque stains (Figure 1b,h) than on less-stained roots (Figure 1e). Since the fluorescence intensities of As Kα and Fe Kβ were correlated, it was concluded that As was associated with Fe minerals (SI Figure S4). These data suggested that Fe-plaque can retard the flow of As toward the root by sorbing it.[26] However, roots without visible Fe-plaque were also found in flooded soils.[36,52] Scattered localization of As and Fe on these roots (Figure 1e,f) was also observed by Seyfferth et al.[36] and Smith et al.[52] Although Fe-plaque did not cover the entire exterior of the root, scattered deposits of Fe hydroxide were able to sequester As.

A significant portion of As on the Fe-plaque was present in the form of As(III) when the soil matrix was anaerobic (Figure 1b). Liu et al.[35] showed that the presence of Fe-plaque can act as a barrier for As(V) absorption by roots while enhancing As(III) absorption. Therefore, the Fe-plaque may not function as a barrier to As absorption by the rice roots grown in flooded soils.

Even when the soil matrix was anaerobic, an oxidative reaction potentially occurred in the rhizosphere due to the release of oxygen and/or oxidants by the roots.[14,53] Liu et al.[31] indicated the predominant presence of As(V) over As(III) in the Fe-plaque of mature roots after harvest. In samples collected during rice growth and panicle exertion, 72% of the As was in the form of As(III) when the Eh of the soil matrix was −90 mV. In contrast, only 46% of the As was in the form of As(III) at an Eh of +213 mV (Figure 1h). Under anaerobic conditions, As(V) is not be dissolved in the soil solution;[4,6] therefore, the As(V) migration to the root from the soil matrix is limited. Thus, the proportion of As(III) on the root surface should become greater than that of soil matrix when the flow of As(III) is retarded at the root−soil interface. The As speciation around the roots was similar to that of the soil matrix, which implied a portion of the As(III) was oxidized to As(V) around the root.[29] In addition, the proportions of As(III) on the Fe-plaque around the roots were similar when the roots were at a similar depth from the soil surface regardless of the development of aerenchyma in the root (SI Figure S6). In contrast, Seyfferth et al.[36] reported that As(III) was dominant on the root tip and that As(V) was dominant in the Fe-plaque of matured roots near the soil surface. The oxygen concentration decreased vertically with soil depth.[41,54] Thus, the speciation of As on the Fe-plaque was likely affected by the Eh of the soil matrix in addition to the root maturity.

Previous studies showed that the formation of Fe-plaque was more pronounced on matured roots that were near the water−air interface.[36,40] Oxygen concentration near the root, which is related to ROL of the roots and depth from the soil surface, controls the formation of Fe-plaque, thereby influencing As sequestration and oxidation around the root. In this study, Fe-plaque did not noticeably cover the root portions of active solute uptake when soil matrix was anaerobic. The absence of Fe-plaque would allow for As absorption from roots (SI Figure S7a), which corresponds to the findings reported by Seyfferth et al.[36]

Transitions from Anaerobic to Aerobic Conditions. The floodwater in rice fields is drained approximately 10 days before harvest. Following drainage, anaerobic soil gradually becomes aerobic, beginning at the soil surface.[41] Although many roots had little staining under the flooded conditions, most of the roots were stained by Fe-plaque 1 month after harvest (SI Figure S3b). In the well-drained soil, the As associated with the Fe-plaque was mainly in the form of As(V); however, As(III) remained on the Fe aggregates in the soil matrix (Figure 2b, SI Table S3). Since the proportions of As(III) and As(V) in the Fe-plaque and soil matrix were similar under flooded conditions (Figure 1b, SI Table S3), the larger proportion of As(V) on the Fe-plaque after harvest suggested that the shift to aerobic conditions was faster around the root. This predominance of As(V) on the Fe-plaque was previously observed for roots after harvest.[31,38]

In contrast to well-drained soil, As(III) remained on the Fe-plaque around the roots collected from poorly drained soils after harvest (Figure 2h). The shift from anaerobic to aerobic conditions near the root channels surrounded by poorly drained soils should be slower than the shift in well-drained soils. Greater ROL during the late stage of rice growth[15] and the supply of O_2 through the root channel after drainage should result in a greater difference between the redox conditions of the root surface and the soil matrix. Different redox conditions potentially led to larger differences in the proportion of As(III) on the Fe-plaque and soil matrix after harvest compared to 1 week after panicle exertion. However, contrasting results showing decreased O_2 concentrations in the rhizosphere during the late rice growth stage have also been reported.[13,40] Schmidt et al.[40] demonstrated that decreased O_2 concentrations resulted in the disappearance of Fe-plaque staining. After subsequent drainage, Fe hydroxide was deposited around the root again.[40] These results indicated that Fe-plaque formation was controlled by the O_2 concentration around the root. Our results suggested that As speciation in the rhizosphere was affected by the redox condition of the soil matrix, which influenced the O_2 concentration in the rhizosphere.

Sequestration of As in Fe Mottles. In the typical management of a paddy field in Japan, rice roots and shoots are incorporated into the soil in the winter so that soil aeration is enhanced in the plow layer. The soil matrix in the plowed layer becomes aerobic while the subsurface soil matrix remains anaerobic (based on the positive reaction of the subsoil with an α,α'-dipyridyl solution in the field). The shrinkage of dead roots results in the formation of pores that can transport O_2 and create an aerobic zone around the root channels. In this study, we provided direct evidence that the As is scavenged by Fe mottles originating from Fe-plaque around the roots in the paddy field (Figure 3).

Frommer et al.[38] classified the concentric accumulation of elements around the roots into three patterns: thin roots with large As/Fe ratios, thin roots with increased Fe and As toward the soil matrix, and thick root channels with an increased As/Fe ratio toward the outer rim of the Fe enrichment zone in addition to the appearance of Mn concretions near the root channel. The diameter of the root channel in tubular Fe mottles observed in this study was similar to the thick roots reported by Frommer et al.[38] However, the elemental distribution patterns were different. In the Fe mottle formed in the plowed layer, As was localized around the root channel and the ratio of As/Fe decreased toward the outer rim of the Fe mottle. When the soil was flooded, As was localized at the root−soil interface (Figure 1b,e,h). The root taken 1 month after drainage also had As localization at the root−soil interface (Figure 2b, f). Consequently, the concentric distribution pattern of As in the tubular Fe mottle likely reflected the accumulation of As around the roots that grew in the continuously flooded soil for approximately 2 months. The contrasting distribution patterns of As between our results and those of Frommer et al,[38] who collected samples from a field with periodic flooding and drying with repeated irrigation, potentially resulted from the different redox cycle frequencies.

In the tubular Fe mottle found in the subsurface layer where the soil matrix was not well aerated, the proportion of As(III) was greater in the outer rim of the mottle relative to the inner portions of the mottle (Figure 3g−i). This trend resulted from the greater oxygen supply to the root channel than that to the soil matrix. In addition to As speciation, the composition of the Fe minerals was influenced by the redox environment. The slow oxidation of Fe(II) at circumneutral pH is a prerequisite for the formation of lepidocrocite.[55] Thus, the occurrence of lepidocrocite (Figure 3f, SI Figure S9) indicated that the shift from anaerobic to aerobic conditions in the outer rim of the mottle was slow. Lepidocrocite was not detected in the inner layer, instead, ferrihydrite, poorly crystallized Fe hydroxide and goethite were the dominant Fe minerals near the inner part of the mottle. The oxidation process in the inner portion of the mottle from the subsurface layer was too fast to result in lepidocrocite formation due to the oxygen supplied from the root channel.

■ ENVIRONMENTAL IMPLICATIONS

In order to decrease As concentrations in rice grains, rice should be grown without flooding.[11] However, cultivating rice under aerobic conditions can result in greater Cd concentrations in rice grains,[10] weed invasion, and decreased yield and quality. In comparison to rice cultivated under continuously flooded conditions, rice cultivated under intermittent flooding and drainage had lower As concentrations.[10] Since the oxidative reaction occurs faster than the development of strongly reductive condition,[41] the temporal drainage associated with intermittent irrigation management may be effective for developing local aerobic conditions around roots in addition to the oxygen supplied through the aerenchyma. We showed that draining floodwater resulted in a faster shift to aerobic conditions near the roots with Fe-plaque than in the soil matrix. Thus, the immobilization of As due to aeration after drainage can be faster in the rhizosphere. The variable distribution and speciation of As with the Fe-plaque/mottles under different redox conditions were potentially affected by microscale redox fluctuations. The different oxidation rates around the roots and the soil matrix with various redox gradients should be further studied to determine the most effective water management strategy for simultaneously decreasing the As and Cd concentrations in rice grains.

■ ASSOCIATED CONTENT

Supporting Information

Additional information for soil and rice root characterization, μXRF maps, μXANES spectra, μXRD patterns is included. This information is available free of charge via the Internet at http://pubs.acs.org/

■ AUTHOR INFORMATION

Corresponding Author

*Tel: +81-29-838-8315; fax: +81-29-838-8315; e-mail: nyamag@affrc.go.jp.

Notes

The authors declare no competing financial interest.

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